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RESEARCH ARTICLE

The chiggerflea *Hectopsylla pulex* (Siphonaptera: Tungidae): infestation on *Molossus molossus* (Chiroptera: Molossidae) in the Central Andes of Colombia

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ABSTRACT. Some species of mastiff bats, *Molossus* Geoffroy, 1805, inhabit human shelters such as houses and barns. Among them, the Pallas's mastiff bat, *Molossus molossus* Pallas, 1766, is the most common species in South America. There are a few studies on this bat in Colombia, mostly on colony size, diet, ectoparasite records, and activity patterns in the Andean and Caribbean regions. Here, we provide information on the prevalence of chiggerfleas, *Hectopsylla pulex* (Haller, 1880), on *M. molossus*, along with molecular data on the flea, and its distribution in Colombia. In addition, we describe the size and sex ratio of the infested bat colony, located in the central Andes of Colombia. The bat colony was represented by ca. 45 individuals, of which 33 were captured. The colony had more females (25 individuals) than males (8 individuals). A total of four Pallas's mastiff bats had chiggerfleas, *H. pulex*, most of which were attached to the bat's faces and ears. The composition of the colony (sex ratio) and the observed activity times match those reported for other colonies of the species in Colombia. The cytochrome oxidase subunit 1, and the 12S rRNA mitochondrial gene obtained from *H. pulex* represent the second and first available sequences for the species, respectively. The level of infestation of individuals in the colony was low, similar to that observed in other South American countries, such as Brazil. Finally, the new locality is the seventh confirmed and the highest elevational record of *H. pulex* in Colombia.

KEY WORDS. Colony, distribution, ectoparasites, sex ratio, urban ecosystems.

INTRODUCTION

Molossus Geoffroy, 1805 comprises one of the most diverse groups of bats in the southern Nearctic and Neotropical regions (Loureiro et al. 2018). Species in this genus are distributed from southeastern United States southward to southern Argentina, and some on the Caribbean islands (Miller 1913, López-González and Presley 2001). Traits commonly used to distinguish *Molossus* are the presence of medium-sized ears, incisors aligned with the canines, and a well-developed cranial crest (Eger 2008). The Pallas's mastiff bat, *Molossus molossus* Pallas, 1766, is one of the most common species in the genus, with a wide geographic distribution (Lindsey and Ammerman 2016). In Colombia it is considered a common species in the lowlands of Andean and Caribbean cities, where roosts may be found in the roofs of houses (Alberico et al. 2005, Sampedro et al. 2007, Ramírez-Chaves et

al. 2008). Information on colony sizes in Colombia is variable (Alberico et al. 2005, Sampedro et al. 2007, Ramírez-Chaves et al. 2008) but up to 200 individuals living in urban houses have been documented (Ramírez-Chaves et al. 2008). There is also very little information on the ectoparasites of this bat in Colombia (e.g., Hastriter and Méndez 2000).

An ectoparasite observed on individuals of molossid bats: *Eumops*, *Molossus*, *Nyctinomops* and *Tadarida* (Méndez 1977, Hastriter and Méndez 2000, Esbérard 2001, Hastriter et al. 2014) is the chiggerflea *Hectopsylla pulex* (Haller, 1880), which has been recorded in Argentina, Bolivia, Brazil, Colombia, Chile, Ecuador, the United States, Panama, Peru and Venezuela (Hastriter et al. 2014). Most of the observations or collection records of these parasites were made in colonies of *M. molossus*. However, it has been also observed infesting noctilionids: *Noctilio albiventris* Desmarest, 1818; phyllostomids: *Desmodus rotundus* (E. Geoffroy, 1810), *Glossophaga soricina* (Pallas, 1766), *Leptonycteris yerbabuenae* Martínez & Villa-R., 1940, *Phyllostomus hastatus* (Pallas, 1767); and vespertilionids: *Eptesicus brasiliensis* (Desmarest, 1819), *Histiotus velatus* (I. Geoffroy, 1824), and *Myotis nigricans* (Schinz, 1821) (Hastriter and Méndez 2000, Hastriter et al. 2014). Generally, females of *H. pulex* are attached to the host, perhaps due to their reproductive requirements (i.e., maturation of eggs), whereas male chiggerfleas feed and subsequently abandon the host, and generally are associated with colony guano (Méndez 1977, Hastriter and Méndez 2000). In Colombia, *H. pulex* is poorly known with only few published records, the most recent obtained around 1970 (Tamsitt 1970, Méndez 1977, Marinkelle and Grose 1981, Hastriter and Méndez 2000).

Here, we present novel information on the proportion of males to females infested with *H. pulex*, as well as the prevalence and molecular identification of this chiggerflea in colonies of Pallas's Mastiff bats in the Central Cordillera (1,991 m of elevation), of Colombia.

MATERIAL AND METHODS

In June 2019, a colony of *M. molossus* was located on the roof of a rural house at "Hacienda La Graciela (05°18'23.6"N, 75°29'38.3"W, 1991 m of elevation), Vereda Alegrías", Municipality of Aranzazu, Department of Caldas, Colombia. In December 2019, using two mist nets arranged in those places where bats had been observed emerging, we captured specimens, recorded the number of males and females, their reproductive status, and infestation with ectoparasites. For the identification of the bats, we used taxonomic keys for the identification of Neotropical bats (Eger 2008, Díaz et al. 2016). Furthermore, we confirmed the identifications following the cranio-dental measurements of Loureiro et al. (2018), using a 0.01 mm precision Mitutoyo digital caliper.

We removed the ectoparasites using entomological forceps. Fleas were preserved in vials in 99% ethanol. The ectoparasites were initially identified as *Hectopsylla* chiggerfleas, then to species with the aid of reviews of the genus and taxonomic keys provided by Hastriter and Méndez (2000), Acosta and Morrone (2003), Blank et al. (2007), and Hastriter et al. (2014). We then confirmed our identifications using the 12S mitochondrial (≈ 340 pb) genes and Cytochrome Oxidase I (COI; ≈ 700 pb), amplified with the primers reported by Beati and Keirans (2001) and Folmer et al. (1994). Vouchers of bats and ectoparasites were deposited in the Collection of Mammals and the Ectoparasites of the Natural History Museum of the University of Caldas (MHN-UCa: 3209–3241), Manizales, Colombia. To update the distribution of *H. pulex* in Colombia, we searched for literature records (Fuller 1942, Tamsitt 1970, Méndez 1977, Marinkelle and Grose 1981, Hastriter and Méndez 2000), and updated the known records distribution with our new records.

RESULTS

We captured 25 females and eight males of *M. molossus*. The estimated colony size, based on direct observations of individuals that were not caught in the mist nets, was 45 individuals. The first individuals to be captured were males (5:53 pm), and later the females emerged from their refuge. Apparently, males and females do not share the same space within the refuge. The last individual was captured at 6:18 pm. We identified the individuals as *M. molossus* based the presence of a long and bicoloured dorsal hair (with the base lighter than the tips), upper incisors elongated with parallel tips, and length of forearm ranging from 36 to 42 mm.

Twelve per cent of all bats were infested with the chiggerflea *H. pulex*. The chiggerfleas were found attached to the head, especially the accessory structures of the ear (Fig. 1). A total of 11 chiggerfleas were obtained from four adult females. The number of fleas per individual ranged from two to four.

The fleas *H. pulex* can be distinguished from similar taxa by the narrow pointed palpus-bearing lobe of the maxilla and within fleas the females are characterized by a unique s-shaped spermathecal; terga and sterna 2–7 short and wide, lateral ends not touching each other in females with distended abdomen and the successive sterna are spaced, so the captured fleas obviously have this abdominal characteristic (Fig. 2; Hastriter and Méndez 2000, Blank et al. 2007). Furthermore, we obtained 99.5% of molecular confirmation with the COI and 12S rRNA genes. GenBank accession numbers for the sequences generated in the present study are MT280760, MT296816 for the COI genes, and MT272335 for 12S rRNA gene.

We found six previously confirmed localities in Colombia for the occurrence of *H. pulex*. These records include one at the departments of Boyacá and Cundinamarca (municipalities of Muzo and Sasaima respectively), and four from Department of Valle del Cauca (municipalities of Ansermanuevo, Cali, and Cartago). All records come from the inter-Andean valleys of the Cauca and Magdalena rivers (Fig. 3).

Figures 1–2. (1) Chiggerfleas *Hectopsylla pulex* attached to the face and ears of the Pallas's mastiff bat *Molossus molossus*; (2) Chiggerflea *Hectopsylla pulex* obtained from a Pallas's mastiff bat *Molossus molossus* in the Central Andes of Colombia.

DISCUSSION

Hectopsylla fleas have rarely been recorded from bats in Colombia. Although the first record of *H. pulex* for the country was made more than 70 years ago (Fuller 1942, parasitizing *Molossus* sp.), the association with bats in Colombia has been documented circumstantially, with reports for at least five species from Molossidae: *Molossus* sp. (Wenzel and Tipton 1966), *Molossus molossus* (Tamsitt 1970, Méndez 1977, Marinkelle and Grose 1981, Hastriter and Méndez 2000), *Molossus currentium* Thomas, 1901 (Hastriter and Méndez 2000); *Cynomops greenhalli* Goodwin, 1958, *Tadarida brasiliensis* (I. Geoffroy, 1824), and *Eumops glaucinus* (Wagner, 1843) (Marinkelle and Grose 1981), Noctilionidae: *Noctilio albiventris* (Méndez 1977, Hastriter and Méndez 2000), and Phyllostomidae: *Desmodus rotundus* and *Glossophaga soricina* (Méndez 1977; Fig. 3). To date, there are no studies on the incidence of this flea on bats on a national scale, however, Tamsitt (1970) reported three fleas on two *M. molossus* in Department of Cundinamarca, and Marinkelle and Grose (1981) reported 24 fleas on 209 bats of four species, with unspecified localities in Colombia.

The comparison of our results with studies conducted in Brazil (Esbérard 2001, Luz et al. 2009) shows certain similarities, such as the preference of fleas to adhere to areas of the face and

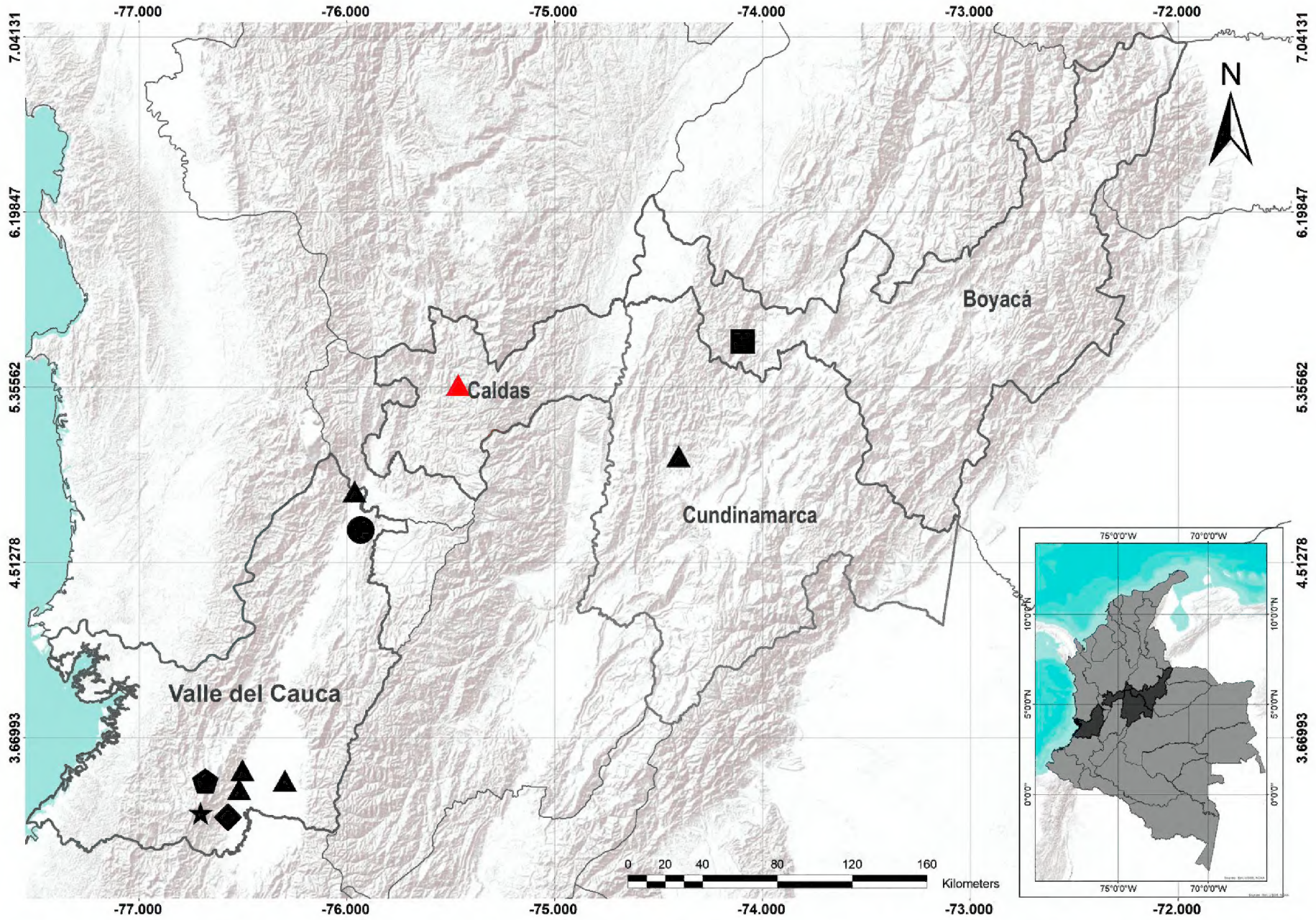

Figure 3. Locality records of *Hectopsylla pulex* in Colombia. The records come from the departments of Valle del Cauca (Tamsitt 1970, Méndez 1977, Hastriter and Méndez 2000), and Caldas (red triangle, this work), in the Cauca River Basin, and from Boyacá (Fuller 1942) and Cundinamarca (Tamsitt 1970) in the Magdalena River Basin. Triangle: *Molossus molossus*, square: *Molossus currentium*, circle: *Molossus bondae* (= *M. currentium*), diamond: *Desmodus rotundus*, star: *Glossophaga soricina*, pentagon: *Noctilio albiventris*. Scale bar: 1 mm.

ears of bats, and a low prevalence of infestation (Esbérard 2001). However, the small size of the evaluated colony, and the low capture effort, prevent us from making more conclusive observations. The individuals obtained in this study probably represent females of *H. pulex*. Although tentative, this identification is supported by the fact that *H. pulex* is the only hectopsyllid representative of the genus that occurs on bats. Males of this species of flea have never been found attached to a host and males have been collected only from bat guano of molossid bats (Méndez 1977, Esbérard 2001, Acosta and Morrone 2003, Luz et al. 2009, Hastriter et al. 2014).

In addition, although the size of the colony is smaller than that observed in other studies carried out in Colombia (see Sampedro et al. 2007, Ramírez-Chaves et al. 2008), it should be noted that the evaluated colony is located at a high elevation in the Andes where the richness and abundance of bats is reduced. As previously shown for the country (Ramírez-Chaves et al. 2008), the colony was mainly composed of females, and bats emerged from the caves before dusk at their first peak of activity.

Finally, the distribution of *H. pulex* in Colombia is not well understood, and probably comprises the Orinoco llanos of Colombia, where previous records without specific locality (i.e., Marinkelle and Grose 1981) were probably obtained. Our records represent the seventh confirmed locality in Colombia, the first in the department of Caldas, and the highest elevation reported for the species in the country.

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